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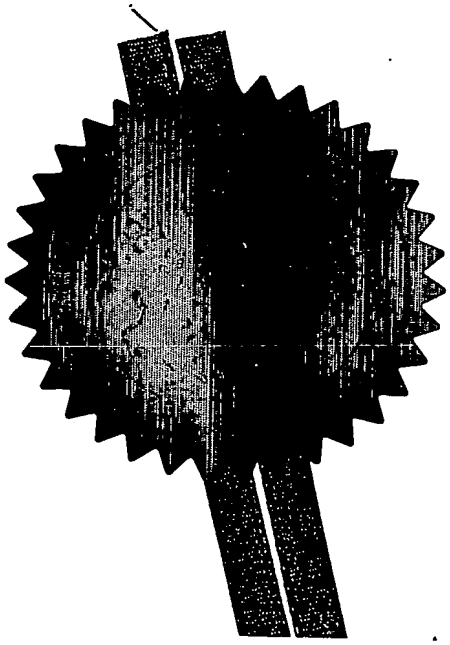

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Dated 5 July 2004

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20JUN03 E816732-1 000019
P01/7700 0.00-0314453.2

P034604GB

2. Patent application number
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0314453.2

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

**JOHNSON & JOHNSON MEDICAL LIMITED**
**Erskine House**
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**Edinburgh**
**United Kingdom**
**EH2 4NH**

Patents ADP number *(if you know it)*

6008031002

If the applicant is a corporate body, give the country/state of its incorporation

**UK (SCOTLAND)**

4. Title of the invention

**ANTIMICROBIAL COMPOSITIONS COMPRISING SILVER**

5. Name of your agent *(if you have one)*

**Carpmaels & Ransford**

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

**43 Bloomsbury Square**
**London**
**WC1A 2RA**

Patents ADP number *(if you know it)*

**83001**

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| Country | Priority application number *(if you know it)* | Date of filing *(day / month / year)* |
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Signature: Carpmaels & Ransford

Date: 19th June 2003

12. Name and daytime telephone number of person to contact in the United Kingdom: **DR. ANTHONY JAMES** **020-7242 8692**

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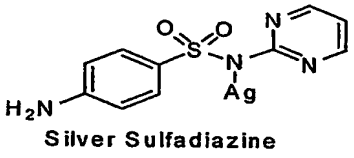
# ANTIMICROBIAL COMPOSITIONS COMPRISING SILVER

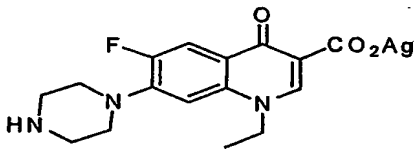
The present invention relates to antimicrobial compositions comprising silver in combination with one or more inhibitors of microbial silver resistance.

The antimicrobial effect of silver has been known for centuries. However, the precise mode of action of silver salts in killing microbes is yet to be established. It is known that silver salts bind with particular avidity to DNA and RNA. Silver salts also bind with particular strength to a variety of organic molecules such as: carboxylic acids, thiols, phenols, amines, phosphates and halogenated compounds. Following binding to proteins, those with enzymic activity are usually deactivated. The oxidative-reductive powers of silver and silver salts must also be reckoned with.

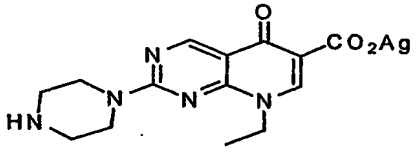
Metallic silver is an effective antimicrobial, whether in the form of thin films, nanoparticles or colloidal silver. Chemical compounds of silver are also useful as antimicrobials. For example, the following complex silver salts are favored for use against sensitive and resistant bacterial strains:



Silver Sulfadiazine



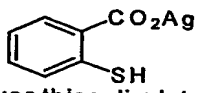
Silver Norfloxacinate



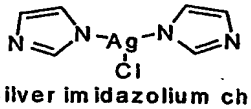
Silver Pipemidate

Kaolin Silver

Silver EDTAate



Silver thiosalicylate



Silver imidazolium chloride

Resistance of certain microbes to silver salts is also known, and is usually attributed either to the presence of a silver-specific binding protein that intercepts the ion, or to two different export proteins. One of the silver export proteins is a

plasmid-coded P-type ATPase (*silP*) and the other is a plasmid-coded membrane potential dependent three-polypeptide cation/proton antiporter (*silCBA*).

It has now been found that certain additives can inhibit or reverse the development of resistance to silver in certain microbes.

Accordingly, in a first aspect, the present invention provides an antimicrobial composition comprising silver and at least one compound which is an inhibitor of microbial silver resistance.

It has further been found that different compounds can inhibit silver resistance in different microorganisms. Accordingly, in order to achieve a broader spectrum of inhibited microorganisms, the antimicrobial composition according to the present invention may comprise at least two different compounds which are inhibitors of microbial silver resistance, in addition to the silver.

Preferably, the inhibitor substance is a substance that, when tested in accordance with the methods described below, inhibits the development of silver resistance in at least one microorganism selected from the group consisting of *Staphylococcus aureus* (ATCC 13709), *Staphylococcus epidermidis* (various CNS strains), *Klebsiella pneumoniae* (ATCC 10031), *Candida albacans* (ATCC 10231), *Escherichia coli* (ATCC 9637 and AG100), *Pseudomonas aeruginosa* (ATCC 27853 and various PSI strains), *Staphylococcus aureus, Vancomycin resistant Enterococci* (VRE F23232, VRE F23245 and VRE 4030101) . Other microorganisms of interest include *Streptococcus Pyogenes*, and certain anaerobic bacteria such as *Bacteroides spp.*, *Prevotella spp*, *porphyromonas spp*, *Peptostreptococcus spp*, and *Clostridia spp.* In some embodiments, the inhibitor compound does not have antimicrobial activity against the target microorganism when it is administered alone, i.e. without silver.

The resistance inhibitors are expected to include molecules that can promote the transport of silver across the cell wall, and/or disrupt the cell wall, of the microorganisms. They may for example function by active transport of the silver

ions, or by modifying the permeability properties of the membrane, for example by physical disruption or by modifying the type and nature of phospholipids present in the membrane. In certain embodiments, the one or more resistance inhibitors are selected from the group consisting of fatty acids, fatty acid esters, fatty alcohols, antioxidants, surfactants, ionophores, enzymes, steroids, essential oils, and mixtures thereof.

Suitable ionophores include the ionophore monensin, which has been shown to transport silver across the basolateral membrane of rainbow trout. Other carboxylic ionophores (e.g. salinomycin and Lasalocid) may help transport silver across bacterial cell membranes thereby reversing the effects of silver resistance.

Enzymes such as Phospholipase A2 and Triacylglycerol hydrolases that modulate the phospholipid component of the membrane may be used, or alternatively enzymes such as papain that act on proteins associated with bacterial cell membranes therefore rendering them more susceptible to silver

Suitable ionic compounds include Calcium thioglycolate, sodium hyaluronate, Azone and related compounds (e.g. alkenylazcycloalkenone derivatives). Suitable Fatty alcohols include Decanol; suitable Aliphatic alcohols include Octanol; suitable Fatty acid esters include Butyl acetate, Glycerol monolaurate, Octyl acetate; suitable Fatty acids include Capric acid Lauric acid Hexanoic acid, Vaccenic acid, Pelargonic acid.

For example, the one or more resistance inhibitors may be selected from the group consisting of fusaric acid, tocopherol and derivatives thereof, retinoids, resveratrol, salicylic acid, dichloroionictonic acid, polyunsaturated fatty acids, green tea extracts, ellagic acid, curcumin, Sorbic acid, Phenoxyethanol, phenethylalcohol, Benzethanium chloride, Zinc Gallate, Polyhexmethylene Biguanide, bisabolo/aloevera, creatine, creatine ascorbate, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, spingomyelin and saponin.

The antimicrobial composition according to the present invention may typically comprise from about 0.01wt.% to about 5wt.% of the one or more resistance inhibiting compounds, based on the dry weight of the composition, for example from about 0.1wt.% to about 3 wt.% of the resistance inhibiting compounds.

The term "silver" herein refers to metallic silver and/or compounds of silver. In certain embodiments the silver comprises metallic silver, in which case it may for example be in the form of a thin film or colloidal particles (nanoparticles) as conventionally known in the art. Alternatively or additionally the silver may be present as a chemical compound or complex, such as those listed above in connection with the prior art.

Preferably, the amount of silver in the compositions according to the present invention is from about 0.01wt% to about 5wt.%, more preferably from about 0.1wt% to about 2wt.%, and most preferably about 0.1wt.% to about 1wt.%, most preferably about 0.3wt.%. Lesser amounts of silver could give insufficient antimicrobial effect. Greater amounts of silver could give rise to antiproliferative effects on wound healing cells.

The silver and the one of more inhibitor compounds may be dispersed in or on any conventional vehicle for antimicrobial compositions, such as a liquid, gel, paste, or solid. In certain embodiments the silver and the one of more inhibitor compounds may be dispersed in or on a solid support material, and preferably they are applied to a surface of such a material.

For example, the solid support material may a woven fabric, a knitted fabric, a nonwoven fabric, or a freeze-dried or solvent-dried sponge. For example, the solid support material may be a fabric comprising textile filaments that are individually coated with a thin film of silver. Especially suitable support materials include charcoal cloth materials of the kind described in GB-A-2206495 and GB-A-2167053, the entire contents of which are incorporated herein by reference. Other suitable support materials include oxidized regenerated cellulose cloths, such as SURGICEL cloth available from Johnson & Johnson Medical Limited. Other

suitable support materials are freeze-dried sponges comprising for example collagen, ORC, alginates, chitosan, or mixtures thereof. Freeze-dried sponges of this type are described for example in EP-A-1153622, the entire contents of which are incorporated herein by reference.

In a second aspect, the present invention provides a wound dressing comprising an antimicrobial composition according to the present invention.

The wound dressing is preferably in sheet form and comprises an active layer of the material according to the invention. The active layer would normally be the wound contacting layer in use, but in some embodiments it could be separated from the wound by a liquid-permeable top sheet. Preferably, the area of the active layer is from about 1cm$^2$ to about 400 cm$^2$, more preferably from about 4cm$^2$ to about 100cm$^2$.

Preferably, the wound dressing further comprises a backing sheet extending over the active layer opposite to the wound facing side of the active layer. Preferably, the backing sheet is larger than the active layer such that a marginal region of width 1mm to 50 mm, preferably 5mm to 20mm extends around the active layer to form a so-called island dressing. In such cases, the backing sheet is preferably coated with a pressure sensitive medical grade adhesive in at least its marginal region.

Preferably, the backing sheet is substantially liquid-impermeable. The backing sheet is preferably semipermeable. That is to say, the backing sheet is preferably permeable to water vapour, but not permeable to liquid water or wound exudate. Preferably, the backing sheet is also microorganism-impermeable. Suitable continuous conformable backing sheets will preferably have a moisture vapor transmission rate (MVTR) of the backing sheet alone of 300 to 5000 g/m$^2$/24hrs, preferably 500 to 2000 g/m$^2$/24hrs at 37.5 °C at 100% to 10% relative humidity difference. The backing sheet thickness is preferably in the range of 10 to 1000 micrometers, more preferably 100 to 500 micrometers. It has been found that such moisture vapor transmission rates allow the wound under the dressing to

heal under moist conditions without causing the skin surrounding the wound to macerate.

Suitable polymers for forming the backing sheet include polyurethanes and poly alkoxyalkyl acrylates and methacrylates such as those disclosed in GB-A-1280631. Preferably, the backing sheet comprises a continuous layer of a high density blocked polyurethane foam that is predominantly closed-cell. A suitable backing sheet material is the polyurethane film available under the Registered Trade Mark ESTANE 5714F.

The adhesive (where present) layer should be moisture vapor transmitting and/or patterned to allow passage of water vapor therethrough. The adhesive layer is preferably a continuous moisture vapor transmitting, pressure-sensitive adhesive layer of the type conventionally used for island-type wound dressings, for example, a pressure sensitive adhesive based on acrylate ester copolymers, polyvinyl ethyl ether and polyurethane as described for example in GB-A-1280631. The basis weight of the adhesive layer is preferably 20 to 250 g/m$^2$, and more preferably 50 to 150 g/m$^2$. Polyurethane-based pressure sensitive adhesives are preferred.

Further layers of a multilayer absorbent article may be built up between the active layer and the protective sheet. For example, these layers may comprise an absorbent layer between the active layer and the protective sheet, especially if the dressing is for use on exuding wounds. The optional absorbent layer may be any of the layers conventionally used for absorbing wound fluids, serum or blood in the wound healing art, including gauzes, nonwoven fabrics, superabsorbents, hydrogels and mixtures thereof. Preferably, the absorbent layer comprises a layer of absorbent foam, such as an open celled hydrophilic polyurethane foam prepared in accordance with EP-A-0541391, the entire content of which is expressly incorporated herein by reference. In other embodiments, the absorbent layer may be a nonwoven fibrous web, for example a carded web of viscose staple fibers. The basis weight of the absorbent layer may be in the range of 50-500g/m$^2$, such as 100-400g/m$^2$. The uncompressed thickness of the absorbent layer may be in the range of from 0.5mm to 10mm, such as 1mm to 4mm. The

free (uncompressed) liquid absorbency measured for physiological saline may be in the range of 5 to 30 g/g at 25°. Preferably, the absorbent layer or layers are substantially coextensive with the active layer.

The wound facing surface of the dressing is preferably protected by a removable cover sheet. The cover sheet is normally formed from flexible thermoplastic material. Suitable materials include polyesters and polyolefins. Preferably, the adhesive- facing surface of the cover sheet is a release surface. That is to say, a surface that is only weakly adherent to the active layer and the adhesive on the backing sheet to assist peeling of the adhesive layer from the cover sheet. For example, the cover sheet may be formed from a non-adherent plastic such as a fluoropolymer, or it may be provided with a release coating such as a silicone or fluoropolymer release coating.

The compositions and dressings according to the present invention are preferably sterile and packaged in a microorganism-impermeable container. Preferably, the sterility assurance level is better than $10^{-6}$. Preferably, the dressing has been sterilized by gamma-irradiation.

The compositions and dressings according to the present invention may further comprise therapeutically effective metal ions other than silver, for example bismuth, copper, nickel, zinc, manganese, magnesium, gold, or mixtures thereof. Preferably, the amounts of such metals are from 0.001 to 10wt.% of the composition or dressing, more preferably from 0.01 to 1wt.% of the composition or dressing. Preferably, the amounts of said other metals is from 10 to 10000ppm, more preferably from about 50 to about 1000ppm in the composition or dressing.

Preferably, the wound dressings according to the present invention are suitable for application directly to a wound surface.

In a further aspect, the present invention provides the use of a composition according to the present invention for the preparation of a dressing for the treatment of wounds. Preferably, the medicament is a wound dressing according

to the present invention. Preferably, the treatment comprises applying the dressing directly to the surface of the wound.

Specific embodiments of the invention will now be described further, for the purpose of illustration, in the following examples:

Reference Example 1

A zone of inhibition method was used to assess the antimicrobial activity of commercially available silver-containing dressings. Discs (6mm) from the impregnated barrier preparations (Acticoat®, Hydrophilic Wound Dressing, Arglaes®, Contreet®-H and Silverlon®) were prepared and placed on lawns of test bacteria that had been seeded onto Oxoid® nutrient agar. Zones of bacterial inhibition were measured daily during the first five days of incubation. Each experiment was performed in triplicate and the zone sizes reported as averages.

Resistance was observed visually by noting growth of individual colonies in the otherwise clear zones of inhibition around the disks. In other cases, resistance was seen to take the form of enhanced growth at the edge of the zone.

Experiments were performed with bacteria obtained from the American Type Culture Collection or from University sources. Strains of bacteria assessed in these studies included *Staphylococcus aureus* (ATCC 13709), *Staphylococcus epidermidis* (various CNS strains), *Klebsiella pneumoniae* (ATCC 10031), *Candida albacans* (ATCC 10231), *Escherichia coli* (ATCC 9637 and AG100), *Pseudomonas aeruginosa* (ATCC 27853 and various PSI strains) and a hospital-derived multiple drug resistant strain of *Staphylococcus aureus* (provided by the Dept of Clinical Microbiology and Pathology) and *Vancomycin resistant Enterococci* (VRE F23232, VRE F23245 and VRE 4030101).

It was found that none of the silver containing dressings was effective against *E. coli* AG100), *E.coli* ATCC9637 and *K. pneumoinae* ATCC 10031).

The yeast *C. albacans* was found to become resistant to silver, in the absence of inhibitor molecules, in almost all cases. This species therefore appears to be a suitable test model for assaying the ability of test molecules to overcome silver resistance.

Silver nitrate and silver sulfadiazine were also examined briefly for comparative purposes. They were inactive against *Escherichia coli* and *Klebsiella pneumoniae*. Activity was seen against *Staphylococcus aureus, Salmonella choleraesuis, Pseudomonas aeruginosa* and *Candida albicans*. Resistance was seen with *Candida albicans*.

Example 1

Tocopherol (Vitamin E) is a powerful antioxidant, neutralizes unstable free radicals, is lipid soluble and exerts its effects by stabilizing biological membranes. As a result of these properties it is implicated in the prevention of a wide range of degenerative diseases including heart disease, cancer, senility and diabetes.

Tocopherol was introduced into each dressing by adding 5ml of a 10µg/ml stock solution. The effect of these treatments on silver resistant colonies was assessed, and the results are summarised in Table 1.

The results demonstrate that tocopherol has no inherent antimicrobial activity, although in one sample some antimicrobial activity against *M. Smegmatis* was observed.

The results also demonstrate that Tocopherol overcomes silver resistance in some bacterial species. Tocopherol reversed silver resistance of C. *albacans* to silver derived from Acticoat, Silverlon and Contreet H in all cases. Tocopherol increased the size of the zones of inhibition against *S. choleraesuis* and *P. aeruginosa* in all dressing. This observation suggests Tocopherol may increase the sensitivity of bacteria to silver, and would be consistent with the molecule having properties that could be used to overcome silver resistance. However, tocopherol was ineffective

at eliminating the ability of S. *aureus* and *M. smegmatis* to become resistant to silver.

Example 2

Resveratrol (trans-3, 5,4'-trihydroxystilbene) is a compound found largely in the skins of red grapes and has historically been used in oriental medicine used to treat diseases of the blood vessels and heart disease. It has antioxidative properties and has been shown to prevent or reduce tumour growth in animal studies by a mechanism involving the inhibition of cyclooxygenase-1 (COX-1), an enzyme that converts arachidonic acid to pro-inflammatory substances that stimulate tumor-cell growth.

Resveratrol was infused into each dressing by adding 5ml of a 10µg/ml stock solution. The effect of these treatments on silver resistant colonies was assessed, and is summarised in Table 2:

It can be seen that Resveratrol demonstrated the same pattern as Tocopherol on resistance. It had no inherent antimicrobial activity, almost completely reversed silver resistance against *C. albacans*, and appeared to increase the sensitivity of *S. choleraesuis* and *P. aeruginosa* to silver (as seen by an increase in the zones of inhibition). However as with Tocopherol, Resveratrol did not eliminate and in some cases appeared to promote the ability of *S. aureus* and *M. smegmatis to* become resistant to silver.

Example 3

Myristic acid is a natural fatty acid that is contained in plant and animal fats and that is also used as an additive in many foods.

Myristic acid was embedded into each dressing by adding 5ml of a 10µg/ml stock solution. The effect of these treatments on silver resistant colonies was assessed, and the results are summarised in Table 3.

The results show that Myristic acid has no inherent antimicrobial activity against any of the strains tested. It did not appear to increase the zones of inhibition when added to silver containing dressings in the absence of obvious signs of silver resistance. However, in four cases were silver resistance was observed against the dressing alone (*C. albacans* against Acticoat, Silverlon and Contreet-H and *S. aureus* against Contreet-H). This data is consistent with that for fusaric acid, and suggests that this molecules may be particularly useful at overcoming silver resistance.

Example 4

Green Tea (Pharmanex®) is present in green tea and is a strong antioxidant. It is sold by a variety of companies as a substance, which will protect against disease by a mechanism involving scavenging of free radicals.

Green Tea (Pharmanex®) was embedded into each dressing by adding 5ml of a 10µg/ml stock solution. The effect of these treatments on silver resistant colonies was assessed, and are summarised in Table 4.

The green tea extract was found to have very unique properties. Although, in five out of eight cases it had no antimicrobial properties on its own it did show antimicrobial activity against *S. aureus*, *M. smegmatis* and *C. albacans*. This made interpretation of the results very difficult as the apparent ability of this molecule to help *C. albacans* overcome silver resistance can partly be explained by its inherent antimicrobial activity against this species. However, one interesting property of this molecule is that although silver or Pharmanex on its own had no antibacterial activity, in this test system, the combination of these molecules appeared to promote antibacterial activity against *K. pneumoniae.*

Example 5

Curcumin is any anticancer drug that exerts its effects by inhibiting protein kinases involved in the G2 phase of the cell cycle. It is also known that it provides a large number of molecules (e.g. pesticides) from gaining entry to cells.

Curcumin was embedded into each dressing by adding 5ml of a 10µg/ml stock solution. The effect of these treatments on silver resistant colonies was assessed, and the results are shown in Table 5.

The results demonstrate that this molecule showed antimicrobial activity against *S. aureus*, *M. Smegmatis*, and *C. albacans*. These observations demonstrate that curcumin has antimicrobial properties in its own right, and although some synergic effects were observed with silver containing dressings against *C. albacans*, this was unlikely due to a mechanism involving the reversal of resistance to silver. It is likely that the pharmaceutical effects, or curcumin, may explain the antimicrobial properties observed here.

The inherent antimicrobial effects of curcumin suggest that this molecule is not suitable for use as a molecule that could increase the sensitivity of bacterial cells to silver or reverse silver resistance.

Example 6

Ellagic acid is a compound found in many fruits such as raspberries. It is reported to inhibit mutations in bacteria and was therefore tested as a potential molecule that would inhibit changed at the molecular (DNA) level that would cause the bacteria to become resistant to silver. However, this molecule was shown to have inherent antimicrobial properties. It did have synergistic effects with silver; however, due to its own antimicrobial properties it was impossible to ascertain the mechanism of this silver resistance.

Example 7

Fusaric acid is a mycotoxin produced by certain molds and fungi. It is known to be a dopamine beta-hydroxylase inhibitor. However, Fusaric acid itself is inactive against human pathogenic bacteria.

The effect of Fusaric acid on the development of silver resistance was studied by a method similar to that of Examples 1 and 2. 5µl of 10mg/ml solution was embedded on a 6mm disc of dressing. The diameter of the zone of inhibition was

measured in mm after incubation for 5 days at 37°C the number of resistant colonies within the Zone of Inhibition was recorded as RC values. R%+ approximate of the area of inhibition covered by resistant colonies. In the fusaric acid studies, five clinically-derived multidrug-resistant strains of *S. epidermidis* were also used. Fusaric acid was inactive by itself against these strains but resistance development/emergence was not seen when fusaric acid was combined with silver preparations. Similar results were seen with five clinically-derived multidrug-resistant strains of *Ps. aeruginosa*. The data obtained for Fusaric acid are shown in Table 6.

The above examples demonstrate that Tocopherol (vitamin E), Resveratrol and Myristic acid have an ability to reverse the silver resistance exhibited by *C. albacans*. In addition, Tocopherol and Resveratrol appear to increase the sensitivity of some types of bacteria to silver but have no effect on the silver sensitivity of other species. Both these molecules were found to be ineffective at reversing silver resistance exhibited by *S. aureus* and *M. smegmatis*, suggesting that both these molecules do not have a universal ability to reverse silver resistance. In contrast, although myristic acid did not increase the size of the zones of inhibition, when applied to dressings of wild type bacteria, it did reverse silver resistance in all four instances when silver resistance was observed. Fusaric acid is a particularly promising compound for the inhibition of silver resistance in a range of pathogenic microorganisms.

The Examples have been described for the purpose of illustration only. Many other compositions and methods falling with the scope of the present invention will be apparent to the skilled reader.

**Table 1**

| Strains | Tocopherol | Acticoat | Tocopherol + Acticoat | Silverlon | Tocopherol + Silverlon | Contreet-H | Tocopherol + Contreet-H |
|---|---|---|---|---|---|---|---|
| S. aureus ATCC 13709 | 0 mm | 15 mm | 15 mm RC=8 | 13 mm | 12 mm RC=4 | 18 mm R=20% | 20 mm RC=14 |
| S. choleraesuis ATCC 9184 | 0 mm | 12 mm | 15 mm | 11 mm | 12 mm | 14 mm | 15 mm |
| P. aeruginosa ATCC 27853 | 0 mm | 11 mm | 16 mm | 11 mm | 12 mm | 13 mm | 15 mm |
| *M. smegmatis* ATCC 607 | 7 mm | 14 mm | 12 mm R=20% | 8 mm | 8 mm | 18 mm | 13 mm R=15% |
| *C. albicans* ATCC 10231 | 0 mm | 13 mm R=20% | 23 mm | 12 mm R=30% | 15 mm | 16 mm R=50% | 30 mm |

**Table 2**

| Strains | Resveratrol | Acticoat | Resveratrol + Acticoat | Silverlon | Resveratrol + Silverlon | Contreet -H | Resveratrol + Contreet-H |
|---|---|---|---|---|---|---|---|
| S. aureus ATCC 13709 | 0 mm | 15 mm | 18 mm RC=13 | 13 mm | 15 mm RC=11 | 18 mm R=20% | 23 mm R=3% |
| S. choleraesuis ATCC 9184 | 0 mm | 12 mm | 13 mm | 11 mm | 12 mm | 14 mm | 16 mm |
| P. aeruginosa ATCC 27853 | 0 mm | 11 mm | 16 mm | 11 mm | 12 mm | 13 mm | 16 mm |
| *M. smegmatis* ATCC 607 | 0 mm | 14 mm | 16 mm R=1% | 8 mm | 13 mm RC=10 | 18 mm | 20 mm R=1% |
| *C. albicans* ATCC 10231 | 0 mm | 13 mm R=20% | 27 mm R=1% | 12 mm R=30% | 21 mm R=3% | 16 mm R=50% | 30 mm R=5% |

**Table 3**

| Strains | Myristic Acid | Acticoat | Myristic Acid + Acticoat | Silverlon | Myristic Acid + Silverlon | Contreet -H | Myristic Acid + Contreet-H |
|---|---|---|---|---|---|---|---|
| S. aureus ATCC 13709 | 0 mm | 15 mm | 13 mm | 13 mm | 10 mm | 18 mm R=20% | 19 mm |
| S. choleraesuis ATCC 9184 | 0 mm | 12 mm | 13 mm | 11 mm | 11 mm | 14 mm | 14 mm |
| P. aeruginosa ATCC 27853 | 0 mm | 11 mm | 14 mm | 11 mm | 11 mm | 13 mm | 14 mm |
| *M. smegmatis* ATCC 607 | 0 mm | 14 mm | 10 mm | 8 mm | 10 mm | 18 mm | 14 mm |
| *C. albicans* ATCC 10231 | 0 mm | 13 mm R=20% | 17 mm | 12 mm R=30% | 17 mm | 16 mm R=50% | 22 mm |

**Table 3**

| Strains | Myristic Acid | Acticoat | Myristic Acid + Acticoat | Silverlon | Myristic Acid + Silverlon | Contreet -H | Myristic Acid + Contreet-H |
|---|---|---|---|---|---|---|---|
| S. aureus ATCC 13709 | 0 mm | 15 mm | 13 mm | 13 mm | 10 mm | 18 mm R=20% | 19 mm |
| S. choleraesuis ATCC 9184 | 0 mm | 12 mm | 13 mm | 11 mm | 11 mm | 14 mm | 14 mm |
| P. aeruginosa ATCC 27853 | 0 mm | 11 mm | 14 mm | 11 mm | 11 mm | 13 mm | 14 mm |
| *M. smegmatis* ATCC 607 | 0 mm | 14 mm | 10 mm | 8 mm | 10 mm | 18 mm | 14 mm |
| *C. albicans* ATCC 10231 | 0 mm | 13 mm R=20% | 17 mm | 12 mm R=30% | 17 mm | 16 mm R=50% | 22 mm |

**Table 4**

| Strains | Green Tea | Acticoat | Green Tea + Acticoat | Silverlon | Green Tea + Silverlon | Contreet -H | Green Tea + Contreet-H |
|---|---|---|---|---|---|---|---|
| S. aureus ATCC 13709 | 10 mm | 15 mm | 16 mm | 13 mm | 13 mm | 18 mm R=20% | 17 mm |
| S. choleraesuis ATCC 9184 | 0 mm | 12 mm | 14 mm | 11 mm | 11 mm | 14 mm | 16 mm |
| P. aeruginosa ATCC 27853 | 0 mm | 11 mm | 13 mm | 11 mm | 12 mm | 13 mm | 15 mm |
| *K. pneumoinae* ATCC 10031 | 0 mm | 0 mm | 7 mm | 0 mm | 7 mm | 0 mm | 0 mm |
| *M. smegmatis* ATCC 607 | 8 mm R=20% | 14 mm | 12 mm R=15% | 8 mm | 9 mm | 18 mm | 13 mm R=15% |
| *C. albicans* ATCC 10231 | 16 mm | 13 mm R=20% | 23 mm | 12 mm R=30% | 18 mm | 16 mm R=50% | 24 mm |

**Table 5**

| Strains | Curcumin | Acticoat | Curcumin + Acticoat | Silverlon | Curcumin + Silverlon | Contreet -H | Curcumin + Contreet-H |
|---|---|---|---|---|---|---|---|
| *S. aureus* ATCC 13709 | 9 | 15 | 13 | 13 | 13 | 18 R=20% | 19 |
| *S. choleraesuis* ATCC 9184 | 0 | 12 | 13 | 11 | 11 | 14 | 14 |
| *P. aeruginosa* ATCC 27853 | 0 | 11 | 13 | 11 | 12 | 13 | 16 |
| M. smegmatis ATCC 607 | 8 | 14 | 11 | 8 | 9 | 18 | 12 |
| C. albicans ATCC 10231 | 9 | 13 R=20% | 18 | 12 R=30% | 15 | 16 R=50% | 23 |

**Table 6**

| Strains | Fusaric Acid | Acticoat | Fusaric acid + Acticoat | Silverlon | Fusaric Acid + Silverlon | Contreet-H | Fusaric acid +Contreet-H |
|---|---|---|---|---|---|---|---|
| E.coli AG100 | 0 mm | 0 mm | 11 mm | 0 mm | 8 mm | 0 | 0 |
| S. choleraesuis ATCC 9184 | 0 mm | 12 mm | 13 mm | 11 mm | 12 mm | 14 mm | 16 mm |
| P. aeruginosa PSA083349-1 | 0 mm | 14 mm | 17 mm | 25 mm | 25 mm | 12 mm | 12 mm |
| P. aeruginosa PSA084459 | 0 mm | 12 R=50mm | 13 mm | 9 mm | 11 mm | 11 mm | 14 mm |
| P. aeruginosa PSA083367-1-1 | 0 mm | 9mm | 17 mm | 8 | 17 mm | 11mm | 14 mm |
| P.aeruginosa | 0 | 12mm R=50% | 14mm | 9mm | 10mm | 12 | 16mm (R=15%) |

CLAIMS

1. An antimicrobial composition comprising silver and at least one compound which is an inhibitor of microbial silver resistance.

2. An antimicrobial composition according to claim 1, comprising at least two compounds which are inhibitors of microbial silver resistance.

3. An antimicrobial composition according to claim 1 or 2, wherein the silver comprises metallic silver.

4. An antimicrobial composition according to any preceding claim, wherein the silver is applied to a solid support material.

5. An antimicrobial composition according to any preceding claim, wherein the one or more resistance inhibitors are selected from the group consisting of fatty acids, fatty acid esters, fatty alcohols, antioxidants, surfactants, ionophores, enzymes, steroids, essential oils, and mixtures thereof.

6. An antimicrobial composition according to any of claims 1 to 4, wherein the one or more resistance inhibitors are selected from the group consisting of monensin, salinomycin, Lasalocid, Phospholipase A2, Triacylglycerol, papain, Calcium thioglycolate, sodium hyaluronate, Azone, Decanol, Octanol, Butyl acetate; Glycerol monolaurate; Octyl acetate, Capric acid, Lauric acid, Hexanoic acid, Vaccenic acid, Pelargonic acid, fusaric acid, tocopherol and derivatives thereof, retinoids, resveratrol, salicylic acid, dichloroionictonic acid, polyunsaturated fatty acids, green tea extracts, ellagic acid, curcumin, Sorbic acid, Phenoxyethanol, phenethylalcohol, Benzethanium chloride, Zinc Gallate, Polyhexmethylene Biguanide, bisabolo/aloevera, creatine, creatine ascorbate, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, spingomyelin and saponin.

7. An antimicrobial composition according to any preceding claim, wherein the composition comprises from about 0.01wt.% to about 5wt.% of silver, based on the dry weight of the composition.

8. An antimicrobial composition according to any preceding claim, wherein the composition comprises from about 0.01wt.% to about 5wt.% of the one or more resistance inhibiting compounds, based on the dry weight of the composition.

9. An antimicrobial composition according to any preceding claim, comprising a solid substrate material incorporating the silver and the one or more resistance inhibiting compounds.

10. An antimicrobial composition according to claim 9, wherein the substrate is a woven fabric, a knitted fabric, a nonwoven fabric, or a freeze-dried or solvent-dried sponge.

11. An antimicrobial composition according to claim 9, wherein the substrate comprises charcoal.

12. An antimicrobial composition according to claim 9, wherein the substrate comprises a material selected from the group consisting of oxidized regenerated cellulose, collagen, chitosan, alginates, and mixtures thereof

13. An antimicrobial composition according to any preceding claim, which is sterile and packaged in a microorganism-impermeable container.

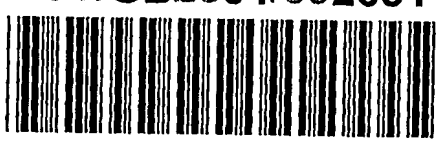
14. A wound dressing comprising an antimicrobial composition according to any one of claims 1 to 13.

15. A wound dressing according to claim 14, which is sterile and packaged in a microorganism-impermeable container.

# ABSTRACT

## ANTIMICROBIAL SILVER COMPLEXES

An antimicrobial composition comprising silver and at least one compound which is an inhibitor of microbial silver resistance. Inhibitor compounds include fusaric acid, tocopherol, resveratrol, and myristic acid. Also provided are wound dressings comprising the inventive compositions.



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